JOURNAL OF BACTERIOLOGY, Feb. 1977, p. 789-795
Vol. 129, No. 2

Printed in U.S.A.

Some Properties of a Membrane-Deoxyribonucleic Acid Complex Isolated from *Bacillus subtilis*

JEFFREY M. HARMON[1] AND HARRY W. TABER*

Departments of Radiation Biology and Biophysics and Microbiology, University of Rochester, Rochester, New York 14642*

Received for publication 23 July 1976

Membrane-deoxyribonucleic acid (DNA) complexes were isolated from *Bacillus subtilis* by affinity for magnesium-Sarkosyl crystals. These complexes (M-bands) contained greater than 80% of the total cellular DNA; little of the remaining portion could be recovered in a secondary isolation. Isotopic labeling of the origin of replication showed this region of the chromosome to be closely associated with the cell membrane. Interruption of protein or DNA synthesis did not result in detachment of the chromosome from the membrane. Interruption of ribonucleic acid synthesis by rifampin resulted in a decreased ability to isolate DNA in the M-band. Analysis of attachment of the chromosome to membrane during the cell and replication cycles indicated that the chromosome is not released from the membrane at any time during the cell cycle.

The in vivo attachment of the bacterial chromosome to the cell membrane was suggested by electron micrographic studies (20) and has since been established by biochemical (8, 21) and genetic (22) analyses. It has been postulated that such attachment should have a functional role in the control of initiation of chromosome replication (11). Indeed, genetic studies in *Bacillus subtilis* (17, 26, 27) and biochemical studies in *Escherichia coli* (7) have indicated that the chromosomal origin is permanently attached to the membrane.

Membrane-deoxyribonucleic acid (DNA) complexes have been isolated from bacteria by isopycnic centrifugation (9) and by electrophoretic separation (16); however, three methods based on sedimentation in sucrose gradients have been most frequently used for the isolation of these complexes: (i) collection of the rapidly sedimenting fraction after gentle osmotic lysis (8); (ii) isolation of compact nucleoids from cells lysed with detergent at 10°C (25); and (iii) the M-band procedure, based on a differential affinity of that portion of the cell membrane to which the chromosome is attached for crystals of Mg^{2+}-Sarkosyl (6, 23). Since we wished to study the unique properties of this membrane region, we chose the M-band fractionation method to obtain membrane-DNA complexes.

We have analyzed M-bands obtained from *B. subtilis* to establish whether such membrane-DNA complexes have properties similar to membrane-DNA complexes isolated by other methods. M-bands have been studied with respect to: (i) attachment of the replicative origin to membrane and (ii) attachment of the chromosome to the membrane during inhibition of macromolecular synthesis and during the cell cycle.

MATERIALS AND METHODS

Bacterial strains. *B. subtilis* strain RB1 (*trpC2*) was originally derived from strain 168. The *dna* initiation mutant, *B. subtilis* 168 *ts-151*, described (13) and kindly provided by Neil Mendelson, was used to construct strain RB334 (*trpC2 ts-151*) by transformation (2).

Growth conditions. Bacteria were cultivated, with aeration, at 30, 37, or 45°C in the minimal salts medium of Anagnostopoulos and Spizizen (1), supplemented with: glucose, 0.5%; sodium glutamate, 0.5%; tryptophan, 50 μg/ml; and methionine, 50 μg/ml. Growth was estimated by following the change in optical density of the culture at 540 nm.

Continuous labeling of DNA. DNA of growing cells was radiolabeled for at least three generations in the presence of 0.5 μCi of [*methyl*-^{3}H]thymidine (New England Nuclear Corp., 2.0 Ci/mmol) or 0.2 μCi of [*methyl*-^{14}C]thymidine (New England Nuclear Corp., 50 mCi/mmol) per ml and 250 μg of deoxyadenosine per ml (Sigma).

Isolation of membrane-DNA fractions. Membrane-DNA complexes (M-bands) were isolated by the method of Tremblay et al. (23). Cells (5×10^9 maximum per sample) were grown and radiolabeled in the appropriate manner. Sodium azide was added to a final concentration of 650 μg/ml, and the cells

[1] Present address: Laboratory of Biochemistry, Naticnal Cancer Institute, National Institutes of Health, Bethesda, MD 20014.

were harvested by centrifugation at 4°C in a Sorvall (model RC2-B) centrifuge at 18,000 × *g*. The cell pellet was resuspended in 2.5 ml of TMK [0.01 M tris(hydroxymethyl)aminomethane - hydrochloride, 0.01 M Mg^{2+}-acetate, 0.1 M KCl, pH 7.0], containing 20% (wt/vol) sucrose to which 0.25 ml of lysozyme (20 mg/ml, Sigma) was added, and incubated for 20 min at 37°C with vigorous shaking to allow removal of the cell wall and the formation of protoplasts. Removal of cell wall was judged complete when >90% of the cell figures appeared as dark spheres under phase microscopy. The protoplast suspension was chilled in an ice bath for 1 min, and then 1.25 ml of 0.3% Sarkosyl (Geigy) in 20% sucrose was added. Lysis of the protoplasts was immediate, as indicated by complete loss of turbidity and increased viscosity of the suspension. After a 15-min incubation to allow crystallization of Mg^{2+}-Sarkosyl to commence, the suspension was carefully layered onto a 37.0-ml, discontinuous 25 to 40% sucrose gradient buffered with TMK and centrifuged for 30 min at 15,000 rpm in a Beckman SW27 rotor. After centrifugation, a narrow band of crystals of Mg^{2+}-Sarkosyl was clearly evident at the interface of the 25 and 40% sucrose layers. Gradients were separated into 1.0-ml fractions using an ISCO model D density gradient fractionator and an ISCO type 540 fraction collector. Alternatively, when only M-band or membrane-free DNA was to be assayed, a syringe fitted with an 18-gauge needle was used to withdraw the appropriate fractions directly from the gradient. Fractions were precipitated in ice-cold 10% trichloroacetic acid. Each precipitate was collected on 0.45-μm membrane filters (Millipore Corp.) and washed with 20 ml of 5% trichloroacetic acid and 10 ml of water. Radioactivity was assayed in toluene-based liquid scintillation fluid in a Beckman model LS-230 liquid scintillation counter.

Synchronization of cells. Populations of synchronized cells were obtained as previously described (3) by a modification of the procedure of Mitchison and Vincent (15). Cells (200 ml) were grown to mid-logarithmic phase, and 160 ml (in 20-ml aliquots) was harvested by low-speed centrifugation. The remaining 40 ml of culture was filtered through a 0.45-μm Millipore filter, and filtrate (conditioned medium) was collected. Each cell pellet was resuspended in 1.25 ml of water, vortexed briefly, and sonicated for 15 s. These cell suspensions were then layered onto 37-ml, 2 to 12% linear sucrose gradients and centrifuged at 2,000 rpm for 12 min. The top 3.0 ml of cells from each gradient was withdrawn with a syringe fitted with an 18-gauge needle and discharged into prewarmed (37°C) conditioned medium (40 ml).

Determination of cell numbers. Samples (0.5 ml) of culture were mixed with 0.5 ml of 0.85% NaCl containing 0.1% NaN_3, sonicated briefly, and counted in a Petroff-Hauser chamber.

Measurement of rate of DNA synthesis. A 50-μl portion of (*methyl*-^{3}H)thymidine (30 μCi/ml) was added to 1.5 ml of culture and incubated for 4 min at 37°C with aeration. A 0.1-ml sample of labeled cells was then pipetted into 1.0 ml of ice-cold trichloroacetic acid containing 0.2 mg of unlabeled thymidine per ml. Acid-insoluble material was assayed for radioactivity as described above.

Inhibition of macromolecular synthesis. Nalidixic acid (Sigma, 50 μg/ml), rifampin (Calbiochem, 200 μg/ml), and chloramphenicol (Sigma, 250 μg/ml) were used to inhibit DNA, ribonucleic acid, and protein synthesis, respectively. Inhibition by each of these drugs at the given concentration was 95% complete within 2 min. Initiation of DNA synthesis was blocked by shifting the temperature-sensitive *dna* initiation mutant RB334 from 30 to 45°C.

RESULTS

Attachment of DNA to membrane. Sarkosyl-membrane-DNA complexes (M-bands) were isolated from cells grown in the presence of [^{3}H]thymidine for three generations as described in Materials and Methods. Fractionation of M-band gradients and measurement of acid-insoluble radioactivity in each fraction showed that more than 80% of the cellular DNA could be isolated in two adjacent fractions (Fig. 1), at the interface of the two sucrose concentrations. Since free DNA has been shown to have little or no affinity for Mg^{2+}-Sarkosyl crystals (6, 23), the DNA in these two fractions was assumed to be membrane bound.

To ascertain the state of the DNA remaining at the top of these gradients, M-bands were prepared as described above. All but the top 4 ml was removed with a syringe, and this 4-ml portion was then refloated over a second 25 to 40% TMK-buffered discontinuous sucrose gradient. Additional Sarkosyl was added to the 4 ml on top of the gradient, and the gradient was centrifuged and fractionated as before. Analy-

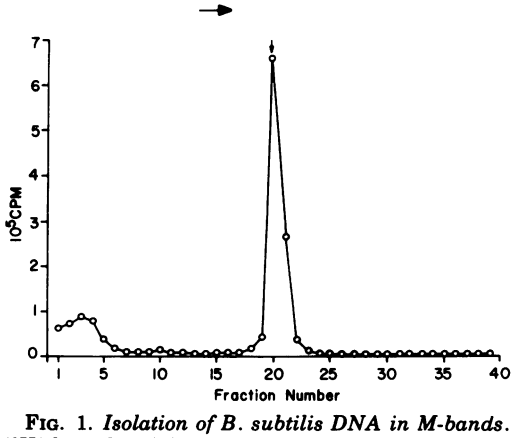

FIG. 1. *Isolation of B. subtilis DNA in M-bands. [^{3}H]thymidine-labeled cells were converted to protoplasts and lysed, and M-bands were formed as described in Materials and Methods. The gradients were fractionated in 1.0-ml samples, and trichloroacetic acid-insoluble radioactivity was measured. Sedimentation was from left to right.*

sis of acid-insoluble material (Fig. 2) revealed that only a small additional amount of DNA could be isolated as M-band material, indicating that the initial separation of membrane-bound from membrane-free DNA had been virtually complete.

Membrane-bound state of replicative origin. Genetic analysis of membrane-DNA complexes contained within rapidly sedimenting fractions has shown that the replicative origin of the chromosome is permanently attached to membrane (17, 26, 27). In these studies the membrane component of the membrane-DNA complex represented a random sampling of the entire plasma membrane. In contrast, membrane-DNA complexes isolated by the M-band method are believed to contain a specific piece of membrane, differentiated from the entire plasma membrane, at least with respect to protein composition (Harmon and Taber, submitted for publication). It was thus of interest to determine whether the specific piece of membrane isolated by this method bears the replicative origin.

To radiolabel the replicative origin of the chromosome, and thus determine the state of its attachment to M-band membrane, it was first necessary to determine the time during the cell cycle when initiation of chromosome replication occurs. Accordingly, synchronous cell populations were obtained by selecting the youngest (smallest) cells after size separation of asynchronously growing cells on linear sucrose gradients, as described in Materials and Methods. These cells were resuspended in conditioned medium. At 10-min intervals both rate of DNA synthesis of the culture and cell number were measured, as described in Materials and Methods. Figure 3a shows the result of such an experiment. Synchrony of cell division was maintained for two and one-half generations; cell divisions occur at 58 and 116 min after resuspension, and each results in almost an exact doubling of cell number. Synchrony of chromosome replication was maintained for a somewhat shorter period of time, becoming asynchronous after two generations. However, initiation of chromosome replication was reproducibly observed to occur at 54 and 110 min after resuspension, or about 4 to 7 min prior to cell division.

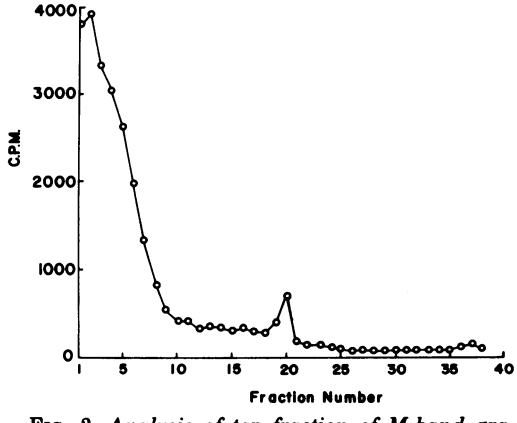

FIG. 2. *Analysis of top fraction of M-band gradients. M-band gradients were prepared as described in Materials and Methods. All but the top 4.0 ml was removed with a syringe. These were then refloated over a second 25 to 40% discontinuous sucrose gradient. The gradient was centrifuged and fractionated as in Fig. 1, and fractions were assayed for radioactivity.*

To insure that the observed synchrony (Fig. 3a) was the result of age selection rather than induction by the techniques used, cells were harvested, resuspended in water, sonicated, and then held in 4% sucrose for 12 min prior to resuspension in conditioned medium. When these cells were assayed for growth and rate of DNA synthesis (Fig. 3b), it was found that both cell division and rate of DNA synthesis were asynchronous, indicating that the result observed in Fig. 3a was obtained through having selected a defined population of cells.

Taking advantage of the fact that initiation of chromosome replication occurs just prior to cell division, cells containing radiolabeled replicative origins were easily obtained. An asynchronous population of cells was pulse labeled with [^{3}H]thymidine (2.0 μCi/ml, 2.0 Ci/mmol) for 6 min. The youngest cells were immediately isolated as in the synchrony experiments described above. These cells had initiated chromosome replication 4 to 7 min previously (the time during which the pulse had been administered) and thus contained chromosomes labeled at or near the replicative origin.

To determine whether the replicative origin was permanently attached to M-band membrane, cells ($\sim 2 \times 10^7$) containing [^{3}H]thymidine-labeled replicative origins were grown in unlabeled conditioned medium for 20 min to allow the chromosomal growing points to move well away from the origin. These cells were then mixed with $\sim 10^8$ cells that had been prelabeled for three generations with [^{14}C]thymidine. This mixture was converted to protoplasts and lysed with Sarkosyl. One-half of the lysate was sheared vigorously by repeated refluxing through a 1.0-ml pipette. The other half was left untreated. Each fraction was centrifuged on M-band gradients; M-band and top fractions were isolated from each gradient. Measure-

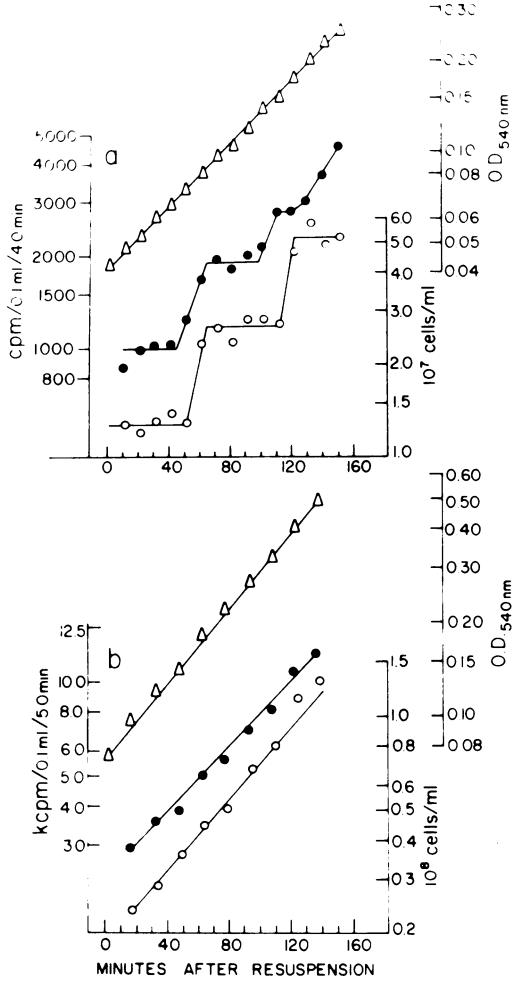

FIG. 3. *Chromosome replication in synchronous cultures of B. subtilis. Cells of uniform small (a) or random (b) size were resuspended in conditioned medium. At 10-min intervals, 1.0-ml samples were removed and incubated with [^{3}H]thymidine (1.0 μCi/ml) for 4 min, at which time 0.1 ml of labeled cells was immediately pipetted into 1.0 ml of ice-cold trichloroacetic acid. The precipitate was assayed for radioactivity* (●). *Turbidity (optical density at 540 nm [OD_{540}]) of the culture* (△) *and cell number* (○) *were also monitored.*

ment of ^{14}C and ^{3}H content in each fraction revealed that whereas only 5% of the ^{14}C continuous label was shear resistant, 25% of the ^{3}H pulse label could not be removed from the membrane by an equivalent amount of shear (Table 1). This result indicates that the replicative origin of the chromosome is preferentially retained in the M-band membrane fraction.

Removal of DNA from membrane. A variety of treatments was used in an attempt to release or detach the chromosome from the membrane. Cells were grown in the presence of nalidixic acid (50 μg/ml) for 10 min or rifampin (200 μg/ml) or chloramphenicol (250 μg/ml) for up to 1 h to block DNA, ribonucleic acid, or protein synthesis, respectively. Of these dugs, only rifampin produced a significant reduction in the DNA content of the M-band fraction (Table 2). We did note, however, that exposure of cells to chloramphenicol for as little as 10 min resulted in a marked decrease in the viscosity of the M-band (data not presented).

The effect of rifampin on the recovery of DNA in the M-band could have been the result of actual detachment of DNA from membrane, or of conformational changes in the chromosome which rendered the DNA more sensitive to shear. Since DNA was isolated as M-band ma-

TABLE 1. *Association of the replicative origin with the M-band*

Treatment	Top fraction		M-band	
	cpm	% Total cpm	cpm	% Total cpm
^{3}H pulse-labeled replicative origin				
Unsheared	1,760	5	35,000	95
Sheared	13,000	75	4,100	25
^{14}C continuous labeled bulk DNA				
Unsheared	23,710	10	201,000	90
Sheared	164,000	90	7,800	5

TABLE 2. *Effect of inhibiting macromolecular synthesis on attachment of DNA to membrane*

Method of inhibition	Time of inhibition (min)	% DNA in M-band[a]
Naladixic acid (50 μg/ml)	10[b]	96
Chloramphenicol (250 μg/ml)	60	99
Rifampin (200 μg/ml)	10	30
	20	28
	45	25
	60	28
Temperature-sensitive initiation[c] (RB334)	60	81

[a] Values represent percentage of untreated control for each experiment averaged over the total number of experiments (at least two).

[b] Nalidixic acid inhibition of DNA synthesis was 95% effective within 1 min. Thus, any direct effect of nalidixic acid on attachment should be seen by 10 min. At longer times, there was some decrease in the amount of DNA in the M-band, probably due to cell death and nonspecific degradation of DNA.

[c] Cells were held at 45°C for 1 h, allowing all rounds for replication to proceed to completion.

terial by virtue of its attachment to membrane (6, 23), it was considered possible that if, as has been suggested for *E. coli* (6, 18), rifampin treatment caused unfolding of the chromosome, the unfolded chromosome would not be carried into the gradient by the Mg^{2+}-Sarkosyl-cell membrane complex without experiencing considerable shear force. This possibility seemed likely in view of the high viscosity of both M-band and top fraction DNA after rifampin treatment.

To test this possibility, two cultures of RB1 were grown to mid-logarithmic phase; one was prelabeled with [^{14}C]thymidine and the other with [^{3}H]thymidine. The ^{14}C-labeled culture was divided into two fractions, one of which was treated with 200 μg of rifampin per ml for 10 min. Protoplasts were prepared from the ^{3}H-labeled culture and from both the rifampin-treated and untreated ^{14}C-labeled cultures. Half of the ^{3}H-labeled protoplasts was mixed with the ^{14}C-labeled rifampin-treated protoplasts, and the other half was mixed with the ^{14}C-labeled rifampin untreated protoplasts. Each preparation was lysed with Sarkosyl, and M-band gradients were prepared. M-band and top fractions from each gradient were isolated, and the percentage of ^{14}C- and ^{3}H-labeled DNA in each M-band was computed. The results (Table 3) indicated that not only did rifampin treatment decrease the ability to isolate DNA from the rifampin-treated cells as M-band material, but also, the presence in the centrifuge tube of DNA from rifampin-treated cells decreased the ability of DNA from untreated cells to be isolated in the M-band fraction. We attribute this result to the increased viscosity of DNA isolated from rifampin-treated cells, thus causing the Sarkosyl lysates to become so viscous that even intact DNA molecules from untreated cells cannot escape into the gradient without experiencing excessive shear force.

Attachment of DNA to membrane was also studied as a function of the cell division cycle. M-bands were isolated from synchronously growing cells, prepared as described in Materials and Methods. Determination of the fraction of DNA that was membrane bound at various times during the cell cycle failed to indicate any significant detachment of DNA from the membrane at any time during the cell cycle (Fig. 4).

The effect of blocking initiation of chromosome replication on the attachment of DNA to membrane was determined using a temperature-sensitive *dna* initiation mutant, strain RB334. When the mutant was grown at the nonpermissive temperature for 1 h to allow all rounds of replication in progress to be completed, there appeared to be no significant decrease in the amount of DNA attachment to membrane (Table 2).

TABLE 3. *Interaction between rifampin-treated and untreated lysates*

Composition of gradient	% ^{3}H-labeled DNA in M-band[a]	% ^{14}C labeled DNA in M-band[a]
^{3}H + ^{14}C (− rifampin)	83	100
^{3}H + ^{14}C (+ rifampin)	37	39

[a] Values computed on the basis of ^{14}C untreated DNA normalized to 100% for each experiment.

DISCUSSION

The membrane fractionation achieved by the M-band procedure seems to be useful for an examination of the role of the cell membrane in the control of chromosome replication. We have studied several of the properties of M-bands isolated from *B. subtilis* and have found them to be similar to those reported for membrane-DNA complexes isolated by other means and in other systems. Two areas deserve further discussion.

(i) Membrane attachment of the chromosomal replicative origin. We have shown that attachment of the replicative origin to membrane is stable to shear forces at a time during the cell cycle when the replication forks are well removed from the origin. Although only 25% of the pulse was retained (Table 1), this may be due to the duration of the pulse, which was approximately 10% of the chromosome replica-

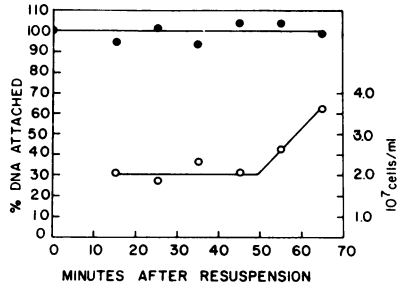

FIG. 4. *Attachment of DNA to membrane during the cell cycle. Cells prelabeled with [^{3}H]thymidine were resuspended for synchronous growth in conditioned medium. Five-milliliter samples were removed at various times and added to 20 ml of unlabeled carrier cells. M-band gradients were obtained as described in Fig. 1. Top and M-band fractions were removed with a syringe and assayed for radioactivity, and the percentage of total activity in the M-band fractions was calculated* (●). *Cell number was measured* (○) *to establish that the culture remained synchronized during the experiment.*

tion time. Since only 5% of the bulk DNA is shear resistant, 50%, at most, of the pulse should have been retained (assuming all shear-resistant DNA is at the origin). However, there are multiple sites of membrane attachment along the length of the chromosome (10), and these account for a portion of the 5% shear resistance of the bulk DNA. Therefore, considerably less than 5% of the chromosome is shear-resistant origin, and, correspondingly, less than 50% of the pulse would be retained in the M-band after shear. Genetic analysis of rapidly sedimenting membrane fractions of *B. subtilis* has yielded similar results (17, 26).

(ii) Detachment of chromosomes from membrane. Interruption of DNA or protein synthesis did not detach the chromosome from its complex with the membrane. Similarly, blockage of chromosome initiation in a temperature-sensitive *dna* initiation mutant had no effect, in agreement with previous reports on the effects of *dna* mutations on membrane-DNA attachment in *B. subtilis* (24). The decrease in M-band-associated DNA isolated from lysates of rifampin-treated cells ("rif lysates") may be due in part to an in vivo, rifampin-induced, conformational change in the chromosome, which is manifested in vitro in increased shear sensitivity. Rif lysate interference with the appearance in M-bands of DNA from untreated lysates reflects the increased viscosity of rif lysates, rendering even intact DNA unable to sediment into M-band gradients without being sheared. This increased viscosity of rif lysates also has been observed in *E. coli* (6). Unfolding of the native *E. coli* chromosomal structure by rifampin inhibition of ribonucleic acid synthesis has been inferred from electron micrographic studies of nuclear bodies (6) and biochemical studies on membrane-free folded nucleoids (18). A recent study (4) of very gently isolated, folded nucleoids from rifampin-treated *E. coli* showed no such effect; however, so-called membrane-associated folded nucleoids (25) were used in this study. Meyer et al. have proposed (14) that this form results from membrane vesicularization and trapping of DNA; if this is so, the state of the chromosomes contained in these vesicles might not affect their sedimentation properties. The properties of Mg^{2+}-Sarkosyl, membrane-DNA complexes isolated from rifampin-treated *B. subtilis* appear, then, to be consistent with the effects of rifampin on chromosomal structure in *E. coli*.

It has been proposed that in *E. coli* termination of chromosome replication is followed by detachment of the chromosome from the membrane if reinitiation is not allowed to take place (12, 25). We have not found this to be the case in *B. subtilis*. Chloramphenicol treatment for up to 1 h, or interruption of initiation in a temperature-sensitive initiation mutant, failed to decrease the amount of DNA present in M-bands. Our study of DNA-membrane complexes during the cell (and replication) cycle also failed to indicate detachment of DNA from the membrane. However, since termination of one round of replication and initiation of the next were coincident events, it is possible that detachment and reattachment occur too rapidly to detect. The suggestion (12) that in the absence of reinitiation termination of chromosome replication results in detachment from membrane was based on the conversion of the membrane-associated form of the folded nucleoid to the membrane-free form upon completion of rounds of chromosome replication. It has been shown that M-bands prepared from membrane-free folded nucleoids contain virtually all of the DNA (5). Since DNA can only be isolated in M-bands by virtue of its attachment to the membrane, even the so-called membrane-free form of the folded nucleoids must contain some membrane (5, 6). We would suggest, therefore, that in the absence of new rounds of DNA replication completion of ongoing replication does not result in detachment of chromosome from the membrane, but rather in a modification in the structure of the chromosome. The sedimentation properties of the membrane-associated folded nucleoid upon termination of chromosome replication provide some support for this contention (19).

Membrane-DNA complexes of *B. subtilis*, isolated by affinity for crystals of Mg^{2+}-Sarkosyl, exhibit the important properties that have been demonstrated for membrane-DNA complexes isolated by this technique from *E. coli*, and by other procedures in both *E. coli* and *B. subtilis*. These properties are (i) possession of the replicative origin as a firmly bound entity and (ii) a conformational response to rifampin. Therefore, such complexes appear to be suitable for examination of the possible role of the cell membrane in the control of chromosome replication.

ACKNOWLEDGMENTS

We are very grateful to H. Eberle and J. Maniloff for their useful criticisms during the preparation of this manuscript.

This work was supported by U.S. Public Health Service grants AI-09093 from the National Institute of Allergy and Infectious Diseases and RR-05002 from the Division of Research Resources and by grant IN-18 from the American Cancer Society. H.W.T. is a holder of Public Health Service Research Career Award K04AI-70,655, National Institute of

Allergy and Infectious Diseases; J.M.H. was supported by Public Health Service training grant GM-01088 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. 81:741-746.
2. **Bettinger, G. E., and F. E. Young.** 1975. Transformation of *Bacillus subtilis:* transforming ability of deoxyribonucleic acid in lysates of L-forms or protoplasts. J. Bacteriol. 122:987-993.
3. **Chatterjee, A. N., H. Taber, and F. E. Young.** 1971. A rapid method for the synchronization of *Staphylococcus aureus* and *Bacillus subtilis*. Biochem. Biophys. Res. Commun. 44:1125-1130.
4. **Dworsky, P.** 1975. A mild method for the isolation of folded chromosomes from *Escherichia coli*. Z. Allg. Mikrobiol. 15:231-241.
5. **Dworsky, P.** 1976. Membrane attachment of folded chromosomes of *Escherichia coli*. Biochem. J. 154:239-241.
6. **Dworsky, P., and M. Schaechter.** 1973. Effect of rifampin on the structure and membrane attachment of the nucleoid of *Escherichia coli*. J. Bacteriol. 116:1364-1347.
7. **Fielding, P., and C. F. Fox.** 1970. Evidence for stable attachment of DNA to membrane at the replication origin of *E. coli*. Biochem. Biophys. Res. Commun. 41:157-162.
8. **Ganesan, A. T., and J. Lederberg.** 1965. A cell-membrane bound fraction of bacterial DNA. Biochem. Biophys. Res. Commun. 18:824-835.
9. **Ivarie, R. D., and J. J. Pène.** 1970. Association of *Bacillus subtilis* chromosome with the cell membrane: resolution of free and bound deoxyribonucleic acid by sedimentation on Renografin density gradients. J. Bacteriol. 104:839-850.
10. **Ivarie, R. D., and J. J. Pène.** 1973. Association of many regions of the *Bacillus subtilis* chromosome with the cell membrane. J. Bacteriol. 144:571-576.
11. **Jacob, F., S. Brenner, and F. Cuzin.** 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. 28:329-348.
12. **Jones, N. C., and W. D. Donachie.** 1974. Protein synthesis and the release of the replicated chromosome from the cell membrane. Nature (London) 251:252-253.
13. **Mendelson, N. H.** 1972. Deoxyribonucleic acid distribution in *Bacillus subtilis* independent of cell elongation. J. Bacteriol. 111:156-162.
14. **Meyer, M., M. A. DeJong, C. L. Woldringh, and N. Nanninga.** 1976. Factors effecting the release of folded chromosomes from *Escherichia coli*. Eur. J. Biochem. 63:469-475.
15. **Mitchison, J. R., and W. S. Vincent.** 1965. Preparation of synchronous cultures oy sedimentation. Nature (London) 205:987-989.
16. **Olsen, W. L., H.-G. Heidrich, P. H. Hofschneider, and K. Hannig.** 1974. Deoxyribonucleic acid-envelope complex isolated from *Escherichia coli* by free-flow electrophoresis: biochemical and electron microscope characterization. J. Bacteriol. 118:646-653.
17. **O'Sullivan, M. A., and N. Sueoka.** 1972. Multifork attachment of the replicative origins of a multifork (dichotomous) chromosome in *Bacillus subtilis*. J. Mol. Biol. 69:237-248.
18. **Pettijohn, D. E., and R. Hecht.** 1973. RNA molecules bound to the folded bacterial genome stabilize DNA folds and segregate domains of supercoiling. Cold Spring Harbor Symp. Quant. Biol. 38:31-41.
19. **Ryder, O. A., and D. W. Smith.** 1975. Properties of membrane-associated folded chromosomes of *E. coli* related to initiation and termination of DNA replication. Cell 4:337-345.
20. **Ryter, A., and F. Jacob.** 1964. Etude au microscope électronique de la liason entre moyau et mésosome chez *B. subtilis*. Ann. Inst. Pasteur Paris 107:384-400.
21. **Smith, D. W., and P. C. Hanawalt.** 1967. Properties of the growing point region in the bacterial chromosome. Biochim. Biophys. Acta 144:519-531.
22. **Sueoka, N., and W. G. Quinn.** 1968. Membrane attachment of the chromosome replication origin in *Bacillus subtilis*. Cold Spring Harbor Symp. Quant. Biol. 33:695-705.
23. **Tremblay, G. Y., M. J. Daniels, and M. Schaechter.** 1969. Isolation of a cell-membrane-DNA-nascent-RNA complex from bacteria. J. Mol. Biol. 40:67-76.
24. **Villiani, G., G. Mastromei, and S. Riva.** 1974. Membrane attachment of the chromosome in *Bacillus subtilis* temperature sensitive in DNA replication. Nucleic Acids Res. 1:1775-1782.
25. **Worcel, A., and E. Burgi.** 1974. Properties of a membrane attached form of the folded chromosome of *Escherichia coli*. J. Mol. Biol. 82:91-105.
26. **Yamaguchi, K., and H. Yoshikawa.** 1973. Topography of chromosome membrane junction in *Bacillus subtilis*. Nature (London) New Biol. 244:204-206.
27. **Yamaguchi, K., and H. Yoshikawa.** 1975. Association of the replication terminus of the *Bacillus subtilis* chromosome to the cell membrane. J. Bacteriol. 124:1030-1033.